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Wesley B. Ames
FOLEY & LARDNER
P.O. Box 80278
San Diego, CA 92138-0278

EXAMINER	
BERTAGNA, ANGELA MARIE	
ART UNIT	PAPER NUMBER
1637	

SHORTENED STATUTORY PERIOD OF RESPONSE	MAIL DATE	DELIVERY MODE
3 MONTHS	03/22/2007	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

If NO period for reply is specified above, the maximum statutory period will apply and will expire 6 MONTHS from the mailing date of this communication.

Office Action Summary	Application No.	Applicant(s)
	10/813,693	TABOR ET AL.
	Examiner	Art Unit
	Angela Bertagna	1637

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on 07 December 2006.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) 1,11,24 and 124-169 is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) 1,11,24 and 124-169 is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☒ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on 07 November 2003 is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Remarks

1. This application has been re-assigned to Examiner Angela Bertagna in Art Unit 1637 whose correspondence information appears at the end of this Office Action.

Continued Examination Under 37 CFR 1.114

2. A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on January 4, 2007 has been entered. Claims 1, 11, 24, and 124-169 will be examined on the merits.

Specification

3. The disclosure is objected to because of the following informalities: The continuity data presented on the first page of the specification should be updated to reflect the status of parent application 09/480,878.

Appropriate correction is required.

Claim Rejections - 35 USC § 112 – 2nd paragraph

4. The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 1, 11, and 124-163 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 1, 11, and 124-163 are indefinite, because claims 1, 11, 124-127, 157, 158, and 160-163 recite the phrase "at least about." The phrase "at least about" is indefinite, because it renders the minimum required level of amplification completely unclear. See also MPEP 2173.05(b), which states, "....the court held that claims reciting "at least about" were invalid for indefiniteness where there was close prior art and there was nothing in the specification, prosecution history, or the prior art to provide any indication as to what range of specific activity is covered by the term "about." Amgen, Inc. v. Chugai Pharmaceutical Co., 927 F.2d 1200, 18 USPQ2d 1016 (Fed. Cir. 1991)."

Claims 143-146 are further indefinite because they recite the phrase "less than about". The phrase "less than about" is indefinite, because it is entirely unclear what temperature is "less than about 60° C", "less than about 50° C", "less than about 45° C", and "less than about 40° C."

Claim Rejections - 35 USC § 102

5. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

6. Claims 1, 11, 124-128, 130, 131, 137, 138, 141-148, 151, 152, 156-159, 166, and 168 are rejected under 35 U.S.C. 102(b) as being anticipated by Nakai (The Journal of Biological Chemistry (1993) 268(32): 23997-24004; newly cited) as evidenced by Biswas et al. (Biochemical Journal (2004) 379(Pt 3): 553-562; newly cited).

Regarding claims 1 and 166, Nakai teaches a method of amplifying a template DNA molecule comprising:

(a) incubating the template DNA molecule with a reaction mixture comprising a DNA polymerase and at least one accessory protein at a constant temperature to produce amplified products (page 23998, "Bacteriophage Mu DNA Replication in Vitro" (page 23997, column 2 teaches that the E. coli extract used in this method includes a helicase, a primase, and DNA polymerase); see also page 24002 and Figure 7, where Nakai teaches isothermal amplification using a mixture comprising T7 DNA polymerase and accessory proteins)

wherein production of the amplified products does not require exogenously added primers and the template DNA molecule does not have a terminal protein covalently bound to either 5' end (neither amplification method taught by Nakai includes exogenous primers or a terminal protein)

and wherein the method is performed under conditions such that the amplification is exponential and results in at least about 10-fold amplification of the template (see page 24003, column 1).

Regarding claims 11, 156, and 168, Nakai teaches a method of amplifying a template DNA molecule comprising:

incubating the template DNA molecule with an in vitro reaction mixture comprising a DNA polymerase, a helicase, and a primase at constant temperature to produce amplified products (page 23998, "Bacteriophage Mu DNA Replication in Vitro" (page 23997, column 2 teaches that the E. coli extract used in this method includes a helicase, a primase, and DNA polymerase)),

wherein the method is performed under conditions such that under conditions such that the amplification is exponential and results in at least about 10-fold amplification of the template (see page 24003, column 1),

and wherein the method does not require the addition of exogenous primers (neither amplification method taught by Nakai includes exogenous primers).

Regarding claims 124-128, and 157-159, the isothermal amplification methods taught by Nakai are exponential and result in at least 10-fold amplification (page 24003, column 1). As noted above, the phrase "at least about" renders the instant claims 124-127 completely unclear as to the minimum level of amplification required, and therefore, the teachings of Nakai anticipate these claims.

Regarding claims 130 and 131, Nakai teaches replication using T7 DNA polymerase (see page 24002 and Figure 7).

Regarding claims 137 and 138, Nakai teaches that the accessory protein is a helicase or a primase (see page 23997, column 2, where Nakai teaches that the E. coli extract used in the amplification method described on page 23998, column 1 contains a primase and helicase).

Regarding claims 141 and 142, Nakai teaches that the mixture further includes a single-stranded DNA binding protein (see Figure 7 and page 24002, where Nakai teaches inclusion of

the T7 gene 2.5 protein in the amplification reaction mixture). Nakai also teaches on page 23997, column 2, that the E. coli extract used in the amplification method described on page 23998, column 1 contains DnaC protein. This protein binds single-stranded DNA, as evidenced by Biswas et al. (Biochemical Journal (2004) 379(Pt 3): 553-562) (see abstract).

Regarding claims 143-147, Nakai teaches amplification at 37°C (page 23998, column 1 and Figure 7 legend).

Regarding claims 148, 151, and 152, Nakai teaches that the reaction mixture further comprises an ATP regenerating system that includes phosphocreatine and creatine kinase (page 23998, column 1).

Claim Rejections - 35 USC § 103

7. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

8. Claims 24, 160-164, and 169 are rejected under 35 U.S.C. 103(a) as being unpatentable over Nakai et al. (The Journal of Biological Chemistry (1993) 268(32): 23997-24004; newly cited) as evidenced by Biswas et al. (Biochemical Journal (2004) 379(Pt 3): 553-562; newly cited) in view of Tabor et al. (The Journal of Biological Chemistry (1989) 264(11): 6447-6458; cited on IDS; hereafter "Tabor I") and further in view of Bernstein et al. (Proceedings of the National Academy of Sciences, USA (1988) 85: 396-400; cited on IDS) and further in view of Tabor et al. (Journal of Biological Chemistry (1987) 262(33): 16212-16223; cited on IDS; hereafter "Tabor II").

Nakai teaches a method of isothermal amplification utilizing T7 proteins (see page 24002 and Figure 7).

Regarding claims 24 and 169, the method of Nakai comprises incubating a DNA template molecule in an in vitro reaction mixture comprising a wild-type T7 DNA polymerase, a 56-kDa form of a gene 4 protein from bacteriophage T7 (an accessory protein that has helicase activity), and a single-stranded binding protein from T7 (gene 2.5 protein) at a constant temperature to produce amplified products (see page 24002 and Figure 7). This method does not require the addition of exogenous primers, and the template does not have a terminal protein attached to the 5' end.

Regarding claims 160-164 and 169, amplification method taught by Nakai is exponential and results in at least 10-fold amplification (page 24003, column 1).

The method of Nakai differs from the methods of the instant claims in the following ways: (1) Nakai does not teach that the reaction mixture includes a mixture of two polymerases from bacteriophage T7, where one polymerase is reduced in exonuclease activity relative to the wild-type protein, (2) Nakai teaches use of the 56 kDa form of the T7 gene 4 protein rather than the claimed 63 kDa form, and (3) Nakai teaches use of a single-stranded binding protein from bacteriophage T7 rather than a single-stranded binding protein from E. coli.

Tabor I teaches wild-type and variant forms of T7 DNA polymerase (see abstract).

Regarding claims 24 and 169, Tabor teaches a modified form of T7 polymerase that has reduced exonuclease activity (see page 6447, column 1). Tabor teaches that, unlike wild-type T7 DNA polymerase, the modified form can initiate strand-displacement synthesis at nicks, and thus, can be stimulated by T7 helicase. Tabor also teaches that the modified polymerase can incorporate nucleotide analogs, unlike the wild-type enzyme. Tabor states that these properties of the modified enzyme "make it useful for DNA sequence analysis (page 6447, column 2)."

Bernstein compared the 56 kDa and 63 kDa forms of the T7 gene 4 protein (see abstract). Regarding claims 24 and 169, Bernstein teaches that the 7 kDa region absent from the 56 kDa form of the gene 4 protein is required for primase activity (see abstract and page 398).

Tabor II measured the properties of wild-type T7 DNA polymerase. Tabor II teaches that E. coli single-stranded binding protein "stimulates both the rate of elongation and the processivity of the gene 5 protein-thioredoxin complex (abstract)." See also Figure 9 and Table

IV on page 16221, where Tabor II reports an elongation rate up to seven times greater in the presence of E. coli single-stranded binding protein.

It would have been prima facie obvious for one of ordinary skill in the art at the time of invention to apply the teachings of Tabor I, Bernstein, and Tabor II to the isothermal amplification method taught by Nakai. As noted above, Tabor taught that the exonuclease-deficient form of T7 DNA polymerase possessed useful properties absent in the wild-type form, namely the ability to initiate strand displacement synthesis at nicks and also incorporate nucleotide analogs (page 6447, column 1). An ordinary practitioner would have been motivated by these teachings of Tabor I to additionally include the modified T7 DNA polymerase in the reaction mixture taught by Nakai in order to enhance amplification of nicked templates or obtain the ability to directly monitor synthesis by analog incorporation. An ordinary practitioner would also have been motivated to substitute the 63 kDa form of the T7 gene 4 protein, as suggested by Bernstein, in order to obtain the primase activity required for lagging strand synthesis. An ordinary practitioner would also have been motivated to include an E. coli single-stranded binding protein in the reaction mixture, since Tabor II expressly taught that this protein increased the elongation rate and processivity of the T7 polymerase/thioredoxin complex used by Nakai (see abstract). Finally, regarding the claimed levels of amplification (claims 160-164), an ordinary practitioner would have recognized that the reaction conditions in the method resulting from the combined teachings of Nakai, Tabor I, Bernstein, and Tabor II could be optimized to in order to maximize the level of amplification. As noted *In re Aller*, 105 USPQ 233 at 235:

> More particularly, where the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation.

Routine optimization is not considered inventive and no evidence has been presented that the selection of specific reaction parameters was other than routine or that the results should be considered unexpected in any way as compared to the closest prior art. Therefore, the methods of the instant claims 24, 160-164, and 169 are prima facie obvious in view of the combined teachings of Nakai as evidenced by Biswas, Tabor I, Bernstein, and Tabor II.

9. Claims 124-127, 157, and 158 are rejected under 35 U.S.C. 103(a) as being unpatentable over Nakai et al. (The Journal of Biological Chemistry (1993) 268(32): 23997-24004; newly cited) as evidenced by Biswas et al. (Biochemical Journal (2004) 379(Pt 3): 553-562; newly cited).

Nakai teaches the method of claims 1 and 11, as discussed above.

Nakai teaches that the above isothermal amplification method results in at least 10-fold amplification of the initial template (page 24003, column 1). As discussed above, the teachings of Nakai anticipate the method of claims 124-127, 157, and 158, because these claims do not clearly define the minimum required level of amplification. However, Nakai does not teach that the amplification method described above results in 100-10,000,000-fold amplification.

It would have been prima facie obvious for one of ordinary skill in the art at the time of invention to modify the reaction conditions taught by Nakai in order to enhance the level of amplification. An ordinary practitioner would have recognized that result-effective reaction

variables such as temperature, enzyme concentrations, and incubation times could be optimized in order to maximize the level of amplification. As noted *In re Aller*, 105 USPQ 233 at 235:

> More particularly, where the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation.

Routine optimization is not considered inventive and no evidence has been presented that the selection of specific reaction parameters was other than routine or that the results should be considered unexpected in any way as compared to the closest prior art. Therefore, the methods of the instant claims 124-127, 157, and 158 are prima facie obvious in view of the teachings of Nakai as evidenced by Biswas.

10. Claims 129, 132-136, and 165 are rejected under 35 U.S.C. 103(a) as being unpatentable over Nakai et al. (The Journal of Biological Chemistry (1993) 268(32): 23997-24004; newly cited) as evidenced by Biswas et al. (Biochemical Journal (2004) 379(Pt 3): 553-562; newly cited) in view of Tabor et al. (The Journal of Biological Chemistry (1989) 264(11): 6447-6458; cited on IDS; hereafter "Tabor I").

Nakai teaches the method of claim 1, as discussed above.

Regarding claim 133, the T7 polymerase taught by Nakai has an exonuclease activity of about 5,000 units per milligram of protein (see Table IV of Tabor I on page 6455).

Regarding claim 165, Nakai teaches a method for amplifying a template DNA molecule comprising incubating the template molecule with a reaction mixture comprising a DNA polymerase and at least one accessory protein at a constant temperature to produce amplified products (see page 24002 and Figure 7). The method of Nakai does not require the addition of

exogenous primers, and the template molecule does not have a terminal protein attached to the 5' end.

Nakai does not teach that the reaction mixture includes two forms of T7 DNA polymerase, where one form has a wild-type level of exonuclease activity and the other form has a reduced level of exonuclease activity.

Tabor I teaches wild-type and variant forms of T7 DNA polymerase (see abstract).

Regarding claims 129, 132, and 165, Tabor I teaches a modified form of T7 polymerase that has reduced exonuclease activity (see page 6447, column 1). Tabor teaches that, unlike wild-type T7 DNA polymerase, the modified form can initiate strand-displacement synthesis at nicks, and thus, can be stimulated by T7 helicase. Tabor also teaches that the modified polymerase can incorporate nucleotide analogs, unlike the wild-type enzyme. Tabor states that these properties of the modified enzyme "make it useful for DNA sequence analysis (page 6447, column 2)."

Regarding claims 133 and 134, Tabor I teaches that the wild-type T7 DNA polymerase has about 5,000 units of exonuclease activity per milligram of protein, whereas the variant form has less than 50% of the wild-type exonuclease activity (see Table IV on page 6455).

It would have been prima facie obvious for one of ordinary skill in the art at the time of invention to apply the teachings of Tabor I to the isothermal amplification method taught by Nakai. As noted above, Tabor I taught that the exonuclease-deficient form of T7 DNA

polymerase possessed useful properties absent in the wild-type form, namely the ability to initiate strand displacement synthesis at nicks and also incorporate nucleotide analogs (page 6447, column 1). An ordinary practitioner would have been motivated by these teachings of Tabor I to additionally include the modified T7 DNA polymerase in the reaction mixture taught by Nakai in order to enhance amplification of nicked templates or obtain the ability to directly monitor synthesis by analog incorporation. Regarding the claimed wild-type to variant DNA polymerase ratios (claims 135 and 136), an ordinary practitioner would have recognized that this result-effective variable could be optimized to in order to obtain the desired results. As noted *In re Aller*, 105 USPQ 233 at 235:

> More particularly, where the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation.

Routine optimization is not considered inventive and no evidence has been presented that the selection of specific polymerase concentrations was other than routine or that the results should be considered unexpected in any way as compared to the closest prior art. Therefore, the methods of the instant claims 129, 132-136, and 165 are prima facie obvious in view of the combined teachings of Nakai as evidenced by Biswas and Tabor I.

11. Claims 139 and 140 are rejected under 35 U.S.C. 103(a) as being unpatentable over Nakai et al. (The Journal of Biological Chemistry (1993) 268(32): 23997-24004; newly cited) as evidenced by Biswas et al. (Biochemical Journal (2004) 379(Pt 3): 553-562; newly cited) in view of Bernstein et al. (Proceedings of the National Academy of Sciences, USA (1988) 85: 396-400; cited on IDS).

Nakai teaches the isothermal amplification method of claim 1, as discussed above.

Nakai teaches that the amplification reaction comprises the 56 kDa form of the gene 4 protein from bacteriophage T7 rather than the 63 kDa form.

Bernstein compared the 56 kDa and 63 kDa forms of the T7 gene 4 protein (see abstract). Regarding claims 139 and 140, Bernstein teaches that the 7 kDa region absent from the 56 kDa form of the gene 4 protein is required for primase activity (see abstract and page 398).

It would have been prima facie obvious for one of ordinary skill in the art at the time of invention to substitute the 63 kDa form of the T7 gene 4 protein taught by Bernstein for the 56 kDa form taught by Nakai. As noted above, Bernstein taught that the 7 kDa region absent in the 56 kDa form was required for primase activity (abstract, page 398, and Table 2). An ordinary practitioner would have been motivated by these teachings of Bernstein to substitute the 63 kDa form of the T7 gene 4 protein for the 56 kDa form taught by Nakai, in order to obtain the primase activity required for lagging strand synthesis. Therefore, the combined teachings of Nakai as evidenced by Biswas and Bernstein result in the method of the instant claims 139 and 140.

12. Claim 149 is rejected under 35 U.S.C. 103(a) as being unpatentable over Nakai et al. (The Journal of Biological Chemistry (1993) 268(32): 23997-24004; newly cited) as evidenced by Biswas et al. (Biochemical Journal (2004) 379: 553-562; newly cited) in view of Dickinson et al. (Journal of Cell Science (1983) 60: 355-365; newly cited).

Nakai teaches the method of claim 1, as discussed above.

Nakai does not teach that the reaction mixture further includes a nucleotide diphosphokinase.

Dickinson teaches the nucleotide diphosphokinase is required for DNA replication in yeast (summary on page 355).

It would have been prima facie obvious for one of ordinary skill in the art at the time of invention to include a nucleotide diphosphokinase in the isothermal amplification reaction mixture of Nakai. Since Dickinson taught that this enzyme was required for DNA replication, an ordinary practitioner would have been motivated to further include this enzyme in the reaction mixture of Nakai in order to improve the ability of the in vitro system to synthesize DNA. Since purified forms of this enzyme were commercially available at the time of invention, an ordinary an ordinary practitioner would have expected a reasonable expectation of success in incorporating this essential enzyme into the reaction mixture of Nakai. Therefore, the method of claim 149 is prima facie obvious in view of the combined teachings of Nakai and Dickinson.

13. Claim 150 is rejected under 35 U.S.C. 103(a) as being unpatentable over Nakai et al. (The Journal of Biological Chemistry (1993) 268(32): 23997-24004; newly cited) as evidenced by Biswas et al. (Biochemical Journal (2004) 379: 553-562; newly cited) in view of Peller (Biochemistry (1977) 16(3): 387-395; newly cited).

Nakai teaches the method of claim 1, as discussed above.

Nakai does not teach that the reaction mixture further includes an inorganic pyrophosphatase.

Peller investigated the role of pyrophosphate hydrolysis during in vitro synthesis of bacteriophage T7 DNA (see abstract). Peller sought to develop a thermodynamic explanation for the observation that nucleic acids generated by cell-free in vitro synthesis systems often produced products at the small end of the size spectrum (page 387, column 1). Peller stated, "The achievement of both very high molecular weights and sharply peaked size distributions in polynucleotides synthesized in vitro will require coupling to inorganic pyrophosphatase action as in vivo (see abstract)."

It would have been prima facie obvious for one of ordinary skill in the art at the time of invention to include an inorganic pyrophosphatase in the isothermal amplification reaction mixture of Nakai. As noted above, Peller expressly taught that synthesis of nucleic acids of high molecular weight requires inorganic pyrophosphatase activity (see abstract). An ordinary practitioner would have been motivated by these teachings of Peller to further include this enzyme in the reaction mixture of Nakai in order to improve the ability of the in vitro system to synthesize high molecular weight (i.e. full-length) DNA. Since purified forms of this enzyme were commercially available at the time of invention, an ordinary an ordinary practitioner would have expected a reasonable expectation of success in incorporating this essential enzyme into nucleoside diphosphokinase into the reaction mixture of Nakai. Therefore, the method of claim 150 is prima facie obvious in view of the combined teachings of Nakai and Peller.

14. Claims 153 and 154 are rejected under 35 U.S.C. 103(a) as being unpatentable over Nakai et al. (The Journal of Biological Chemistry (1993) 268(32): 23997-24004; newly cited) as

evidenced by Biswas et al. (Biochemical Journal (2004) 379: 553-562; newly cited) in view of Engler et al. (The Journal of Biological Chemistry (1983) 258(18): 11197-11205; cited on IDS).

Nakai teaches the method of claim 1, as discussed above.

Nakai does not teach that the reaction mixture further includes T7 DNA ligase.

Engler teaches a method for conducting lagging strand synthesis using an in vitro T7 replication system (see abstract). Engler teaches that T7 DNA ligase activity is required for successful lagging strand synthesis (see abstract). Engler stated, "All steps in the replication of a lagging strand have been coupled in a model system that catalyzes the formation of covalently closed, circular, double-stranded DNA molecules using single-stranded viral DNA as template. A combination of four bacteriophage proteins, gene 4 protein, Form II of T7 DNA polymerase, gene 6 exonuclease, and DNA ligase, can accomplish this overall reaction (abstract)."

It would have been prima facie obvious for one of ordinary skill in the art at the time of invention to include T7 DNA ligase in the isothermal amplification reaction mixture of Nakai. As noted above, Engler expressly taught that DNA ligase was required for lagging strand synthesis during in vitro replication (see above). An ordinary practitioner would have been motivated by these teachings of Engler to include T7 DNA ligase in the reaction mixture of Nakai in order to more effectively replicate the double-stranded template used in the reaction (see Figure 7 of Nakai). Since Engler taught a method for the purification of T7 DNA ligase (page 11198, column 2), an ordinary practitioner would have had a reasonable expectation of success in adding T7 DNA ligase to the reaction mixture of Nakai. Therefore, the methods of claims 153 and 154 are prima facie obvious in view of the Nakai as evidenced by Biswas in view of Engler.

15. Claim 155 is rejected under 35 U.S.C. 103(a) as being unpatentable over Nakai et al. (The Journal of Biological Chemistry (1993) 268(32): 23997-24004; newly cited) as evidenced by Biswas et al. (Biochemical Journal (2004) 379: 553-562; newly cited) in view of Jarvis et al. (The Journal of Biological Chemistry (1990) 265(25): 15160-15167; newly cited).

Nakai teaches the method of claim 1, as discussed above.

Nakai does not teach that the reaction mixture further includes an additive selected from the group consisting of dextran, potassium glutamate, and DMSO.

Jarvis studied the effect of including high molecular weight polymers such as dextran or polyethylene glycol (PEG) on the stability and processivity of the T4 DNA replication complex (abstract and page 15161). Jarvis reported that PEG and dextran stabilized the DNA replication complex, and thereby, indirectly increased processivity of the polymerase (see Table 1 on page 15162). Jarvis stated, "Although the processivity of the polymerase alone is not directly effected by the addition of such polymers to the solution, macromolecular crowding does significantly stabilize the holoenzyme and thus indirectly increases the observed processivity of the holoenzyme complex (abstract)." Jarvis further stated, "These results suggest that the volume-occupied solution conditions prevalent in vivo can significantly stabilize holoenzyme DNA replication complexes, and thus support high rates and high apparent processivities of DNA synthesis (page 15166)."

It would have been prima facie obvious for one of ordinary skill in the art at the time of invention to include dextran in the isothermal amplification reaction mixture of Nakai. As noted above, Jarvis expressly taught that dextran stabilized the T4 DNA replication complex, and thereby, increased processivity of the polymerase. An ordinary practitioner would have been

motivated by these teachings of Jarvis to include dextran in the reaction mixture taught by Nakai in order to stabilize the T7 DNA replication complex and thereby improve polymerase processivity with the ultimate result being increased amplification efficiency and yield. An ordinary practitioner would have expected a reasonable level of success in doing so, since the Jarvis study was conducted in the highly similar T4 DNA replication system and also since Jarvis taught that the crowding effect responsible for the observed stabilization/increased processivity was generally applicable (pages 15161 and 15166). Therefore, the method of claim 155 is prima facie obvious in view of the combined teachings of Nakai as evidenced by Biswas and Jarvis.

16. Claim 167 is rejected under 35 U.S.C. 103(a) as being unpatentable over Nakai et al. (The Journal of Biological Chemistry (1993) 268(32): 23997-24004; newly cited) as evidenced by Biswas et al. (Biochemical Journal (2004) 379(Pt 3): 553-562; newly cited) in view of Tabor et al. (The Journal of Biological Chemistry (1989) 264(11): 6447-6458; cited on IDS; hereafter "Tabor I") and further in view of Bernstein et al. (Proceedings of the National Academy of Sciences, USA (1988) 85: 396-400; cited on IDS).

Nakai teaches a method of isothermal amplification utilizing T7 proteins (see page 24002 and Figure 7).

Regarding claim 167, the method of Nakai comprises incubating a DNA template molecule in an in vitro reaction mixture comprising a wild-type T7 DNA polymerase, a 56-kDa form of a gene 4 protein from bacteriophage T7 (an accessory protein that has helicase activity), at a constant temperature to produce amplified products (see page 24002 and Figure 7). The T7

DNA polymerase used by Nakai has a normal level of exonuclease activity (see Table IV on page 6455 of Tabor I).

Nakai does not teach that the reaction mixture further includes a T7 DNA polymerase with reduced exonuclease activity. Also, although Nakai teaches the inclusion of primase in one embodiment of the method (page 23997-23998, where a primase from E. coli is used), the amplification conducted in the T7 system does not include a primase activity.

Tabor I teaches wild-type and variant forms of T7 DNA polymerase (see abstract).

Regarding claim 167, Tabor teaches a modified form of T7 polymerase that has reduced exonuclease activity (see page 6447, column 1). Tabor teaches that, unlike wild-type T7 DNA polymerase, the modified form can initiate strand-displacement synthesis at nicks, and thus, can be stimulated by T7 helicase. Tabor also teaches that the modified polymerase can incorporate nucleotide analogs, unlike the wild-type enzyme. Tabor states that these properties of the modified enzyme "make it useful for DNA sequence analysis (page 6447, column 2)."

Bernstein compared the 56 kDa and 63 kDa forms of the T7 gene 4 protein (see abstract). Regarding claim 167, Bernstein teaches that the 7 kDa region absent from the 56 kDa form of the gene 4 protein is required for primase activity (see abstract and page 398).

It would have been prima facie obvious for one of ordinary skill in the art at the time of invention to apply the teachings of Tabor I and Bernstein to the isothermal amplification method taught by Nakai. As noted above, Tabor I taught that the exonuclease-deficient form of T7 DNA polymerase possessed useful properties absent in the wild-type form, namely the ability to initiate strand displacement synthesis at nicks and also incorporate nucleotide analogs (page

6447, column 1). An ordinary practitioner would have been motivated by these teachings of Tabor I to additionally include the modified T7 DNA polymerase in the reaction mixture taught by Nakai in order to enhance amplification of nicked templates or obtain the ability to directly monitor synthesis by analog incorporation. An ordinary practitioner would also have been motivated to substitute the 63 kDa form of the T7 gene 4 protein, as suggested by Bernstein, in order to obtain the primase activity required for lagging strand synthesis. Therefore, the method of claim 167 is prima facie obvious over Nakai as evidenced by Biswas in view of Tabor I and Bernstein.

Response to Arguments

17. Applicant's arguments, see page 16, filed November 14, 2006, with respect to: (a) the rejection of claims 1 and 11 under § 102(b) as anticipated by Engler, (b) the rejection of claims claims 1 and 11 under § 102(b) as anticipated by Applegren, and (c) the rejection of claims 1 and 11 under § 102(b) as anticipated by Scherzinger have been fully considered and are persuasive. These references do not teach amplification of at least 10 fold, and therefore, these rejections have been withdrawn.

Applicant's arguments with respect to claim 24 have been considered but are moot in view of the new ground(s) of rejection.

Conclusion

No claims are currently allowable.

The prior art made of record and not relied upon is considered pertinent to applicant's disclosure. Decker et al. (The Journal of Biological Chemistry (1987) 262(22): 10863-10872) teaches an isothermal amplification method that utilizes a primase, a helicase, and a polymerase and results in approximately 10-fold amplification (pages 10863-10864 and Figure 1).

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Angela Bertagna whose telephone number is 571-272-8291. The examiner can normally be reached on M-F, 7:30 - 5.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on 571-272-0782. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

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Angela Bertagna
Examiner, Art Unit 1637
March 15, 2007

amb

JEFFREY FREDMAN
PRIMARY EXAMINER
3/17/07